Preclinical and Clinical Pharmacology Review

BLA 97-0260[1]

Sponsor: IDEC

Product: Rituximab, [redacted] MAb to CD20, IDEC-2BC8

Rituximab is a chimeric (murine and human) monoclonal antibody directed against CD20 antigen, a cell marker found on the surface of normal and malignant B lymphocytes. The (b)(4) [redacted] antibody is an IgG1 kappa immunoglobulin [redacted] While the Fab domain binds to the CD 20 antigen of B-lymphocytes, the Fc doman recruits immune effector cells to provoke B-cell lysis. Mechanisms of cell lysis may include complement-dependent cytotoxicity (CDC) and antibody-dependent cellular cytotoxicity (ADCC). Studies of flow cytometry demonstrate that the antibody binds to the [redacted] component of complement. Other possible mechanism of cytotoxicity towards B-cells include induction of apoptosis and increased sensitivity to the cytotoxic effect of chemotherapeutic agents. The affinity constant of rituximab for the CD20 antigen is 5.2 to 11.0 nM. The CD 20 antigen is located on pre-B and mature B lymphocytes. It is expressed on >95% of all B-cell non-Hodgkin lymphomas. The CD 20 antigen is not found on hematopoietic stem cells, pro-B cells, normal plasma cells or other normal tissues. Upon antibody binding, CD 20 antigen does not internalize nor is it circulate in the plasma.

Tissue Cross-reactivity Study:

The tissue cross-reactivity of the antibody was determined with various human tissues. A limited pattern of tissue reactivity was found. The antibody bond to cells in the bone marrow, peripheral blood, lymph node, white pulp of the spleen and lymphoid follicles of the tonsil. Lymphoid cells which were found in various organ (e.g. small intestines, kidney and stomach) were also reactive. Hematopoietic stem cells, epithelial cells, neuroectodermal tissue (brain and peripheral nerve) and mesenchymal tissues (skeletal and smooth cells, fibroblasts and endothelial cells) were not reactive with the exception of 2 specimens from the large intestine. Thirty per cent of the microglial cells in 1 of 3 specimens of the spinal cord revealed a weak immunoreactivity.

Preclinical Toxicology and Pharmacokinetic Studies:

Single dose, dose escalation study in cynomolgus monkeys:

A single dose, dose escalation study was performed in cynomolgus monkeys (1/sex/group) at various doses (10, 30, 100 mg/kg) given iv. Blood samples were collected at various time points through day 14. Various clinical observations and hematological variables were measured. No

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[1]. Also see Genentech's BLA 97-0244 for manufacturing

toxicity or mortality related to the test article occurred during the course of the study; 1 monkey given 100 mg/kg experienced emesis. A slight decrease in the percentage of lymphocytes was recorded on day 1 after injection. Recovery was observed by day 7 or 14. Various pharmacokinetic endpoints were calculated. Half-life was estimated using a one and two compartment model. Half-life did not appear to be influenced by dose whereas AUC and Cmax were proportionate. Half-life as a reported for a 1-compartment model varied from 58 to 155 hours when time points over 5 to 240 h were used. When a 2-compartment model was used the initial half-life varied from 29 to 60 h and the terminal half-life from 61 to 143 h. Other pharmacokinetic endpoints are cited in the table below as the average of the 2 animals.

Dose, mg/kg	$AUC_{0-\infty}$, μg/ml	Cmax, μg/ml
10	14,171	175
30	57,218	620
100	221,954	1740

Table 1. Pharmacokinetics of single dose study in cynomolgus monkeys.

Multi-dose pharmacokinetic study:

Cynomolgus monkeys were given 4 weekly iv doses of 269 mg/m^2 of the antibody. Peak plasma concentrations ranged from 161 to 386 μg/ml. B lymphocytes were reduced in the peripheral circulation and as well as follicular and non-follicular areas of the lymph nodes. Fourteen days after treatment with the antibody, peripheral blood was depleted of B-cells in approximately 50% of the animals at 4 weeks and 67% of the animals at 8 weeks. Recovery of peripheral B cell count began approximately 2 weeks after the cessation of treatment. Monkeys dosed weekly with 20 mg/kg for 4 weeks or 20 mg/kg for 8 weeks had plasma antibody concentrations of 191 to 303 μg/ml following the first and second infusions. Two monkeys given a single dose iv of 100 mg/kg exhibited plasma levels greater than 1200 μg/ml. Monkeys given weekly administrations developed antibodies to IDEC-C2B8. These antibodies were found to be directed toward the joining region of IDEC-C2B8.

Multiple Dose Toxicity Study in the Cynomolgus Monkey:

Four different groups of monkeys were given IDEC-C2B8 or vehicle as control by intravenous injection to study the toxicity of the antibody. Groups 1 and 2, were given vehicle only; these groups were composed of 1/sex/group, whereas groups 3 and 4 were composed of 3/sex/group. Groups 3 and 4 were given 20 mg/kg of the antibody. Groups 1 and 3 were dosed weekly for 4 consecutive weeks and killed approximately 2 weeks after the last injection. Groups 2 and 4 were dosed weekly for 8 weeks and killed approximately 2 weeks after the last dose. There was no evidence of significant toxicity which was consistently related to the administration of the

antibody with the following exceptions. Emesis was observed in 2 Group 4 females and 1 Group 3 female at 20 mg/kg at of the antibody. No treatment-related effects were found on body weight, hematological endpoints, clinical chemistry or urinalysis. All animals were killed after their last dose. Analysis of 40 tissues collected from each animal revealed little or no white splenic pulp in 2/6 group 3 animals, 4/6 group 4 animals; in addition, lymphoid atrophy was seen in 3/6 group 3 and all group 4 animals due to the pharmacological activity of the antibody. Concurrent with reduction of splenic and lymphatic tissue content, the expression of CD20+ B lymphocytes in the mandibular lymph nodes and spleen of groups 3 and/or 4 animals. Anti-idiotype and anti-isotype monkey antibodies to IDEC-C2B8. The development of antibodies modified both the pharmacological and pharmacokinetic effects of the IDEC-C2B8.

The following toxicology studies[2] were also performed:

1. IDEC AS0069, A Study of IDEC-C2B8 by Single Intravenous Administration in Cynomolgus Monkeys. Monkeys were given 10, 30 or 100 mg/kg (1/sex/group) iv of antibody. No mortality was observed with the exception of 1 instance of emesis in the male monkey at 100 mg/kg. Platelet depletion was noted at 30 and 100 mg/kg. Lymphocytes were deleted in all animals which returned to baseline line levels between 7 to 14 days. Serum concentration of the antibody were proportionate to dose.

2. IDEC AS0055, An Immunopharmacology and Safety Study of Chimeric Anti-CD-20 (IDEC-C2B8) Antibody Administered by Intravenous Injection in Cynomolgus Monkeys. Monkeys were given either 0 (1/sex/group) or 20 mg/kg 3/sex/group), weekly for either 4 or 8 weeks. No mortality or adverse effects were observed other than a decease in B cells.

3. IDEC AS0060A, Determination of Plasma Levels of IDEC-C2B8 Antibody and Monkey Anti-Murine Antibody Responses in Cynomolgus Monkeys Receiving Intravenous Injections of IDEC-C2B8. Samples taken from IDEC study AS005. Antibody levels >40 μg/ml were sustained over a 24 h to 7 day period following 20 mg/kg weekly dosing over 4 weeks.

Pharmacological and Pharmacokinetic Studies of Various Lots and Methods of Manufacture of IDEC-C2B8

Pharmacological and pharmacokinetic comparisons of various lots and methods of manufacture of IDEC-C2B8 were studied using Sprague-Dawley rats and cynomolgus monkeys. No lot or method dependent differences in pharmacokinetics or pharmacology were found. The t1/2 in rats was reported to be about 4 days as a 1-compartment model and about 0.7 days for t1/2α and

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[2] Selected studies are reviewed as determined by their importance. For a complete

about 7 days for t1/2β. While no qualitative differences were revealed by studies conducted to demonstrate consistency of pharmacological and pharmacokinetic responses between methods of manufacture and lot-to-lot variation, some quantitative differences were observed.

Clinical Pharmacokinetics

The pharmacokinetics of IDEC-C2B8 were studied in a number of clinical trials and various pharmacokinetic relationships were revealed. A fundamental conclusion of these studies is that the pharmacokinetics of IDEC-C2B8 are nonlinear at clinically relevant doses due to 1) saturation of elimination mechanisms and 2) interactions between IDEC-C2B8 with tumor cells.

Doses of 10, 50, 100, 250 or 500 mg/m2 were administered as single doses to patients with recurrent B-cell lymphoma. Various pharmacokinetic parameters including area under the curve (AUC), as a measure of overall exposure, observed maximal plasma concentration (Cmax), clearance and terminal half-life were computed. These pharmacokinetic endpoints were observed to decrease with increasing dose in a nonlinear manner. For example when the AUC was divided by the administered dose to standardize the effect of dose, it was found that 500 mg/m2 gave nearly 7 times more exposure than a dose of 50 mg/m2; similarly, for 500 mg/m2 the Cmax was nearly 2 times higher per unit dose than 50 mg/m2. The nonlinear effect of dose on pharmacokinetic endpoints appeared to plateau at doses above 250 mg/m2. Pharmacokinetics were found to be linear with Cmax and AUC increasing with dose. Mean t1/2 was 118 h.

Patients given the antibody as either 125, 250 or 375 mg/m2 iv once weekly for 4 weeks increased their plasma concentrations of the antibody in a nonlinear fashion. Upon the initial infusion, the t1/2 in patients given 375 mg/m2 was 68.1 h with an associated clearance of 0.0459 L/h whereas upon the fourth infusion, plasma t1/2 was 189.9 h and clearance was 0.145 L/h.

Responders were found to have significantly higher serum levels of antibody as compared to nonresponders. The difference between these 2 groups reached statistical significance at various times: prior to the second or fourth infusion, after the fourth infusion at 1 week, and 1 to 3 months after infusion. A marked decline in the median peripheral blood B-cell levels began after the first dose. B-cell recovery was observed approximately 6 months following the completion of treatment. B-cell levels returned to normal between 9 and 12 months following cessation of treatment.

In a study of patients with recurrent B-cell lymphoma weekly, single doses of IDEC-C2B8 were given at 125, 250 or 375 mg/m2 (studies 102-02 and 102-5). An accumulation of drug was observed in the weekly dosing regimen which was predictable given the known pharmacokinetic endpoints such as half-life. The percentage of responders was found to increase with increasing dose administered, and upon repeated treatment, responders were noted to have higher plasma levels of IDEC-C2B8. A likely explanation for the different pharmacokinetics between

responders and non-responders may be due to the interaction between IDEC-C2B8 and tumor cells. Upon an initial infusion of drug, tumor cells are cleared from the responding patient leaving a smaller number of tumor cells available for the uptake of drug upon subsequent infusions. As tumor cells function as routes of drug elimination, the clearance of drug upon successive infusions is decreased. Correlating to the maximum diameter of the largest tumor lesion, as the baseline sum of the product of perpendicular diameter of the 6 largest lesions at baseline, or circulating numbers of B-cells at baseline, revealed an inverse relationship to serum levels of IDEC-C2B8.

When serum levels of drug were examined in relationship to the histologic type of lymphoma, serum levels were found to be lower for type A in comparison to B, C, and D. This pharmacokinetic finding is consistent with the pharmacodynamic relationship between responders and non-responders previously observed.

In summary, the pharmacokinetics of IDEC-C2B8 are nonlinear to dose and significantly influenced by the presence of target cells and extent of the disease burden of patients. Although a relationship exists between patient factors and the pharmacokinetics IDEC-C2B8, it is not of sufficient magnitude to warrant individualization of the dosing regimen.

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